Tumor Engineering: The Use of Synthetic Scaffolds to Study Prostate Tumorigenesis

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Statement of Purpose: Prostate cancer is the most common cancer diagnosis and the second leading cause of cancer death for men in the developed world. The study of a disease as complex as prostate cancer requires a pre-clinical tumor model that can accurately represent disease progression. Tumorigenic events from initiation through metastasis are defined by the local tumor microenvironment. The extracellular matrix (ECM) components of that microenvironment play a particularly critical role in tumor development, and thus it is of clear interest to develop a model system that allows us to capture this interplay. Traditional xenograft models, which primarily consist of subcutaneously injected cells or cells mixed with an exogenous ECM/growth factor matrix prior to injection, fail to adequately control the microenvironmental interactions critical to tumorigenesis. Biomaterial scaffolds provide an opportunity to precisely manipulate the tumor microenvironment in a controlled way, with inert scaffolds having the distinct advantage of tissue development influenced only by the cells within the scaffold. Thus, a tissue engineered tumor model can re-create more accurate representations of cell proliferation, signaling, and cell-matrix interactions. Furthermore, a scaffold-based system can mimic the overall tumor architecture, which features a hypoxic center, some degree of vascularization for material transport, and stiffer mechanical properties. Here, we propose a 3D porous poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel as an inert scaffold for a prostate cancer model. We chose pHEMA due to its well-characterized biocompatibility. Our lab has shown previously that a precisely tuned pore size can be used to enhance angiogenesis *in vivo* within biomaterial implants. Thus, our porous pHEMA system would serve as the basis for an improved prostate cancer tumor model because of its controllable and pro-angiogenic microenvironment. We present the preliminary results of a pilot *in vivo* study where we engineered prostate tumors based on these scaffolds.

Methods: Briefly, sphere-templated pHEMA scaffolds were prepared by polymerizing HEMA around closely-packed, sintered, monodisperse poly(methyl methacrylate) (PMMA) spheres. The polymer was washed with dichloromethane to remove the PMMA beads, leaving a network of interconnected spherical pores. The scaffolds were seeded by capillary force with $1x10^6$ M12 prostate epithelial cells that were then dynamically cultured for five days. The cell suspension was seeded with and without a 1:1 pre-mixture of cells with concentrated Matrigel®. After culture, the scaffolds were implanted subcutaneously into groups of six athymic nude mice, with control groups of unseeded pHEMA scaffolds and standard subcutaneous xenograft injections of 200 µL Matrigel + $1x10^6$ M12 cells. Resulting tumor volume was recorded over twelve weeks using caliper measurements and calculated with the equation $(LxW^2)/2$. Explants from D21 and D84 were fixed in 10% neutral buffered formalin and OCT for histological analysis. Liver, lymph nodes, lung, diaphragm, femur, and spleen were also explanted to examine for potential metastases.

Results: We have demonstrated the ability to seed and culture cells within sphere-templated porous hydrogels. *In vitro*, seeded cells develop a hypoxic core after seven days culture. In our pilot *in vivo* study, we observed tumor growth for scaffolds seeded with and without Matrigel. Tumor growth profiles over time for the seeded materials were statistically equivalent to that seen with the standard Matrigel xenografts (see Fig. 1), but morphological differences in tumor structure such as a larger hypoxic center in the pHEMA tumors were noted. Preliminary immunohistochemical analyses on D21 explants have confirmed the presence of SV40T+ immortalized M12 cells within the scaffolds along with F4/80+ macrophages and PECAM-1+ endothelial cells. Our continuing analysis of the D84 explants is focused on determining differences in tumor structure, microenvironment, and metastatic potential.

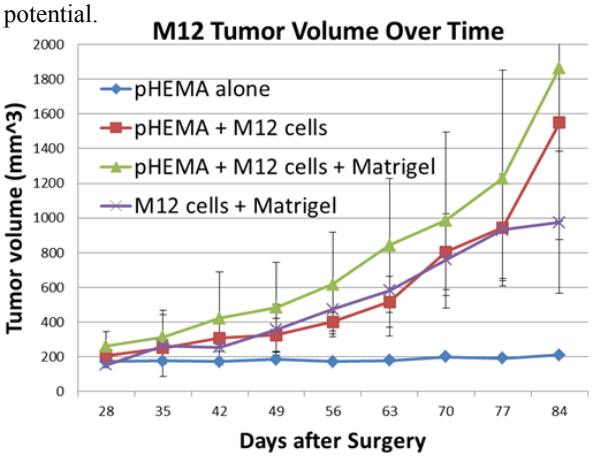

Figure 1: Average volume over time for tumor xenografts derived from implanted pHEMA seeded with M12 cells compared to subQ injected cells + Matrigel

Conclusions: Sphere-templated pHEMA scaffolds are promising for use as a 3D xenograft model to study prostate cancer due to their inert, controllable composition and potential to induce angiogenesis. We have demonstrated solid tumor growth from these materials seeded without exogenous ECM or growth factors. This shows the potential for microenvironmental control while retaining the growth kinetics of standard xenografts. We have also observed stromal cells such as macrophages and endothelial cells infiltrating the material during early tumorigenesis. In an ongoing parallel *in vivo* study, we are analyzing whether microenvironmental changes derived from genetic manipulation of tumor cell laminin subchain expression impact tumor development.

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